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(54) Title: METHOD OF DECREASING THE TOXICITY OF THERAPEUTIC COMPOSITIONS USING THYMOSIN β_4

(57) Abstract

A method of substantially inhibiting normal stem cell proliferation in a mammal including the administration of a normal stem cell proliferation-inhibiting effective amount of Tβ_4 to the mammal. Also, a method of decreasing the toxicity of a toxic therapeutic composition in a mammal including the administration of a normal stem cell proliferation-inhibiting effective amount of Tβ_4 to the mammal.

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METHOD OF DECREASING THE TOXICITY OF THERAPEUTIC COMPOSITIONS USING THYMOSIN β_4

The present invention relates generally to a method of substantially inhibiting normal stem cell proliferation and, more specifically, to a method of decreasing the toxicity of a toxic therapeutic composition in mammals by administering Thymosin β_4 ("Tβ_4") to said mammals.

Description of Prior Art

Myelopoiesis is the process by which cells in the bone marrow of a mammal mature into several different types of myeloid cells including, but not limited to, granulocytes, monocytes, eosinophils and megakaryocytes, where these myeloid cells play important roles in the bodily functions of mammals (e.g., mammals need myeloid cells for proper functioning of their immune systems). Myelopoiesis typically takes 7 days to be completed. On the first day of myelopoiesis, stem cells in the bone marrow are stimulated to proliferate (i.e., undergo an increased number of mitoses), which results in a "proliferative wave" lasting three days. Within the first few days of myelopoiesis, stem cells proliferate and differentiate to form colony forming cells. The maximum point of cell proliferation is generally reached on the third day of myelopoiesis, at which time the cells in the bone marrow are at the promyelocyte stage. Under normal circumstances, the promyelocyte stage is the

latest stage of bone marrow cell differentiation associated with intense proliferation. Following the third day, maturation generally occurs as bone marrow cells become morphologically recognizable and cell proliferation decreases significantly.

$T\beta_4$ is a peptide containing 43 amino acids. The amino acid sequence of $T\beta_4$ is disclosed in U.S. Patent No. 4,297,276, herein incorporated by reference. $T\beta_4$ was highly conserved during evolution. See, Low et al., "Isolation and Structural Studies of Porcine, Ovine and Murine Thymosin B-4 by High-Performance Liquid Chromatography," J. Chromatogr., 301:221 (1984); Spangelo et al., "Biology and Chemistry of Thymosin Peptides: Modulators of Immunity and Neuroendocrine Circuits," Ann. NY Acad. Sci., 496:196 (1987). In fact, total homology exists between murine, rat and human $T\beta_4$. See, Gondo et al., "Differential Expression of the Human Thymosin B-4 Gene in Lymphocytes, Macrophages, and Granulocytes," J. Immunol., 139:3840 (1987); Rudin et al., "Differential Splicing of Thymosin B-4 mRNA," J. Immunol., 144:4857 (1990); Wodnar-Filipowicz et al., "Cloning and Sequence Analysis of cDNA for Rat Spleen Thymosin B-4," Proc. Nat'l Acad. Sci., 81:2295 (1984).

$T\beta_4$ has been found to be present in numerous tissue types in mammals and has also been implicated in a wide variety of cellular and physiological processes including inducing terminal deoxynucleotidyl transferase activity of bone marrow cells, stimulating secretion of hypothalamic luteinizing hormone releasing hormone and luteinizing hormone, inhibiting migration and enhancing antigen presentation of macrophages, and inducing phenotypic changes in T-cell lines in vitro.

Recently, it has been suggested that $T\beta_4$'s N-terminal tetrapeptide, Ser-Asp-Lys-Pro, inhibits the proliferation of myelopoietic stem cells. Guigon et al., "Inhibition of Human Bone Marrow Progenitors by the Synthetic Tetrapeptide AcSDKP," Exp. Hematol, 18: 1112-15 (1990). Furthermore, it has been suggested that this tetrapeptide ameliorates toxicity induced by the administration of toxic compositions such as cyclophosphamide and cytosine arabinoside ("ara-C"). Bogden, et al., "Amelioration of Chemotherapy Induced Toxicity by Cotreatment with AcSDKP, a Tetrapeptide Inhibitor of Hematopoietic Stem Cell Proliferation," Ann. NY Acad. Sci., 628:126-39 (1991).

Nevertheless, toxicity in mammals, particularly humans, resulting from the administration of toxic therapeutic compositions continues to be a major problem facing the medical profession. Often, the use of effective toxic therapeutic compositions (e.g., the use of AZT to treat AIDS and ara-C to treat cancer) must be restricted or even discontinued because of the toxic side effects which these therapeutic compositions have when they are administered to patients. Thus, even though there are some compounds that appear to be somewhat effective in decreasing toxicity resulting from the administration of a toxic therapeutic composition to mammals, there remains a need in the art for effective methods of decreasing the toxicity of toxic therapeutic compositions in mammals.

Summary of the Invention

In accordance with the present invention, a method of substantially inhibiting normal stem cell proliferation in mammals includes administering a

normal stem cell proliferation-inhibiting effective amount of $T\beta_4$ to said mammals.

Further in accordance with the present invention, a method of decreasing the toxicity of a toxic therapeutic composition in mammals includes administering a normal stem cell proliferation-inhibiting effective amount of $T\beta_4$ to said mammals.

Brief Description of the Drawing

Fig. 1 is a graph showing the effect of $T\beta_4$ and its N-terminal tetrapeptide (N4-$T\beta_4$) on ara-C treated mice. The ara-C treated mice were injected with a saline control (+-+), 1 μg $T\beta_4$ (Δ--Δ), 0.1 μg $T\beta_4$ (0---0), 1 μg N4-$T\beta_4$ ($\Delta\bullet\bullet\bullet\Delta$) or 0.1 μg N4-$T\beta_4$ ($0\bullet\bullet\bullet0$). The timing of administration of ara-C and $T\beta_4$/N4-$T\beta_4$ is shown with arrows.

Description of the Preferred Embodiments

The term "$T\beta_4$" as used herein encompasses not only native (i.e., naturally occurring) $T\beta_4$ but also synthetic $T\beta_4$ and recombinant $T\beta_4$ having the amino acid sequence of native $T\beta_4$, amino acid sequences substantially similar thereto, or an abbreviated sequence form thereof, and their analogs and muteins having substituted, deleted, elongated, replaced, or otherwise modified sequences which possess bioactivity substantially similar to that of $T\beta_4$.

The term "toxic therapeutic composition" as used herein refers to therapeutic agents which, when administered to a mammal, can be toxic and to mixtures or combinations thereof. Examples of toxic therapeutic compositions include, but are not limited to: anti-infectious agents such as anti-bacterial agents and anti-viral agents, where examples of suitable anti-

viral agents include, but are not limited to, nucleoside analogs (e.g., dideoxynucleosides such as ddI, ddG, ddC, ddA, ddT and analogs thereof, particularly AZT); anti-cancer agents (e.g., ara-C and methotrexate) and the like.

According to the present invention, methods of substantially inhibiting normal stem cell proliferation are provided. Also provided by the present invention are methods of decreasing the toxicity of a toxic therapeutic composition in mammals. It has been found that these effects can be achieved without substantially affecting the ability of bone marrow cells to mature normally. The methods of the present invention include the administration of a normal stem cell proliferation-inhibiting effective amount of $T\beta_4$ to mammals.

According to a preferred embodiment of the present invention, a normal stem cell proliferation-inhibiting effective amount of $T\beta_4$ is administered to a subject to substantially inhibit normal stem cell proliferation in the subject. In this embodiment, the subject is preferably a human.

According to another preferred embodiment of the present invention, a normal stem cell proliferation-inhibiting effective amount of $T\beta_4$ is administered to a mammal subject to decrease the toxicity of a toxic therapeutic composition in the subject. In this embodiment, the subject is preferably a human.

Toxic therapeutic compositions have greater toxicity against rapidly dividing cells such as cancer cells or stem cells which have been stimulated to proliferate by an active agent such as a chemotherapeutic agent. The administration of a toxic therapeutic composition to such rapidly dividing cells

enables the toxic therapeutic composition to harm or perhaps even kill these cells. Accordingly, inhibiting normal stem cell proliferation so that these normal stem cells are not rapidly dividing decreases the toxicity of the toxic therapeutic composition to these cells.

Without being bound to a particular theory, it is believed that normal stem cell proliferation inhibition in a subject results in the substantial inhibition of the cellular processes of the normal stem cells (e.g., cellular uptake of materials and DNA synthesis) in the subject. Under these circumstances, it would be difficult for a toxic therapeutic composition to be toxic to the non-proliferating normal stem cells of the subject because those cells are not contacting or using any extraneous material (i.e., toxic therapeutic compositions) for their cellular processes. Thus, the toxic therapeutic compositions, because they are not contacting or are not being used by non-proliferating normal stem cells, are less toxic to the subject.

For example, the toxicity of AZT results from normal cells incorporating AZT into their DNA replication process. Such incorporation into the DNA replication process results in incomplete DNA synthesis in those cells (AZT is a "chain terminator") which, in turn, results in toxicity to those cells. Accordingly, where normal stem cells are not proliferating and, therefore, are not synthesizing DNA, nucleoside analogs like AZT cannot be incorporated into the DNA synthesis process and, therefore, cannot be toxic to the normal stem cells.

As can be seen in Table I and the example which follows, $T\beta_4$ is a substantially more effective normal stem cell proliferation-inhibiting compound than its N-

terminal tetrapeptide and is also substantially more effective at decreasing the toxicity of toxic therapeutic compositions like ara-C in mammals than its N-terminal tetrapeptide.

According to preferred embodiments of the present invention, compositions containing $T\beta_4$ may be formulated in a conventional manner for administration by any suitable route. Suitable routes of administration include, but are not limited to, oral, rectal, nasal, topical, vaginal, and parenteral (including subcutaneous, intramuscular, intravenous and intradermal), with oral or parenteral being preferred. It will be appreciated that the preferred route may vary with the condition, age and species of the recipient.

While not essential, it is preferable for $T\beta_4$ to be administered as part of a pharmaceutical formulation. The formulations of the present invention comprise $T\beta_4$ together with one or more pharmaceutically acceptable carriers and optionally with other therapeutic ingredients. The carrier(s) are "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form, e.g., tablets and sustained release capsules, and may be prepared by any suitable pharmaceutical methods.

Such methods include, but are not limited to, the step of bringing into association $T\beta_4$ with the carrier

which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association $T\beta_4$ with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of $T\beta_4$, as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion, etc.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine $T\beta_4$ in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein.

Formulations suitable for topical administration include lozenges comprising $T\beta_4$ in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising $T\beta_4$ in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising $T\beta_4$ to be administered in a suitable liquid carrier.

Formulations suitable for topical administration to the skin may be presented as ointments, creams, gels and pastes comprising $T\beta_4$ and a pharmaceutically

acceptable carrier. A preferred topical delivery system is a transdermal patch containing the ingredient to be administered.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for nasal administration wherein the carrier is a solid include a coarse powder having a particle size, for example, in the range from about 20 to about 500 microns which is administered in the manner in which snuff is taken, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid, for administration, as for example, a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Formulations suitable for vaginal administration may be presented as tampons, creams, gels, pastes, foams or spray formulations containing, in addition to $T\beta_4$, suitable carriers.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may optionally contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use.

Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other suitable agents having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

A proposed daily dose for administration of the compositions in the present invention is a normal stem cell proliferation-inhibiting effective amount of $T\beta_4$, which is in a range from about 0.01 to about 2.0 mg of $T\beta_4$ per kg of body weight of recipient per day (mg/kg/day), preferably from about 0.02 to about 0.2 mg/kg/day.

In accordance with the present invention, $T\beta_4$ can be administered in combination with a therapeutically effective amount of a toxic therapeutic composition. Of course, the acceptable dosage range of the toxic therapeutic composition will depend upon the properties of the toxic therapeutic composition (i.e., the acceptable dosage range will depend upon which toxic therapeutic agent is being administered).

$T\beta_4$ and a toxic therapeutic composition can be administered "in combination" which, as defined herein, includes various schemes designed to administer $T\beta_4$ and a toxic therapeutic composition to a subject, whether or not the toxic therapeutic composition and $T\beta_4$ are administered separately or together, such that the desired dosages of $T\beta_4$ and the toxic therapeutic composition are present in the subject at the same time. Any suitable scheme can be used to administer $T\beta_4$

and a toxic therapeutic composition "in combination" in accordance with the present invention.

The acceptable daily dose of either $T\beta_4$ alone or $T\beta_4$ in combination with a toxic therapeutic composition may be conveniently administered in 1 to 3 doses per day. The precise dose administered will depend on the age, condition and species of the recipient.

The invention having been generally described, the following example is given as a particular embodiment of the invention and to demonstrate the practice and advantages thereof. It is understood that the example is given by way of illustration and is not intended to limit the specification or the claims to follow in any manner.

Example

Effects of $T\beta_4$ and N4-$T\beta_4$ on ara-C Toxicity

$T\beta_4$ and its N-terminal tetrapeptide (N4-$T\beta_4$) were synthesized by the solid-phase procedure described in Wang et al., "Synthesis of Thymosin β_4," Int. J. Protein Res., 18:413 (1981). Cytosine arabinoside (ara-C, Cytosar U-19920, Lot 295AK) was provided as a gift from the Upjohn Company (Kalamazoo, MI).

C3H/HeN male mice, ages 10-11 weeks, were obtained from Jackson Laboratories. All mice were fed on a standard diet, had free access to water, and were housed in an approved animal care facility according to established guidelines. A total of 160 mice were studied in duplicate experiments, with two equal sets of mice being studied on different days to exclude other variables. Mice were divided into four groups. Group I received normal saline and $T\beta_4$ (15 mice). Group II received normal saline and ara-C (29 mice), and served as controls. Group III received ara-C and whole

synthetic $T\beta_4$ (58 mice). Group IV received ara-C and the N-terminal tetrapeptide N4-$T\beta_4$ (58 mice).

Mice were injected intraperitoneally (i.p.) with 5 mg ara-C in a volume of 0.1 ml sterile Dulbecco's phosphate buffered saline (D-PBS) at 0, 7 and 36 hours. Thus, a total of 15 mg of ara-C was injected into the mice. The mice were also injected i.p. at 5 and 34 hours with 0.1 ml D-PBS, N4-$T\beta_4$ in 0.1 ml D-PBS at concentrations of 0.1 μg or 1.0 μg, or $T\beta_4$ in 0.1 ml D-PBS at concentrations of 0.1 μg or 1.0 μg. Survival was recorded daily. Data from both sets of mice were added to form one larger data set. Calculation of z values using a standard confidence test was used to detect differences in survival between test and control groups.

As the endpoint of the assay was survival and evaluation of short-term toxicity, mice were followed for 2 weeks (16 days), after which the survivors were sacrificed per institutional protocol. Cumulative survival is shown in Table I below and illustrated in Fig. 1. No decrement in the number of surviving mice was noted between 8 and 16 days. Statistically significant improvement in survival was noted in groups receiving ara-C plus 0.1 μg $T\beta_4$ or 0.1 μg N4-$T\beta_4$ when compared to controls receiving ara-C alone ($p < 0.01$). This was not seen in groups receiving ara-C plus higher doses of $T\beta_4$ or N4-$T\beta_4$. These data suggest a dose-dependent and bifunctional effect of the administered peptides on abrogation of short-term ara-C toxicity. Appreciable side effects were not seen following administration of either peptide, and no deaths were observed in controls receiving doses of 0.1 μg $T\beta_4$ alone.

Both the N-terminal sequence (N4-Tβ_4) and whole Tβ_4 significantly increased survival over controls ($p < 0.01$). The effect of both peptides was maximal at cumulative doses of 0.2 μg, with less effect at higher doses ($p > 0.05$ for cumulative doses of 2.0 μg). Such bifunctional activity is commonly seen when cytokine activity is analyzed. More importantly, Tβ_4 was more effective than the N-terminal peptide ($p < 0.05$), and this effect was demonstrated in the absence of notable side effects.

While the invention has been described and illustrated with details and references to certain preferred embodiments, those skilled in the art will appreciate that various modifications, changes, omissions, and substitutes can be made without departing from the spirit of the invention.

Table I

Effect of the N-terminal tetrapeptide of $T\beta_4$ ($N4\text{-}T\beta_4$) or $T\beta_4$ on ara-C treated mice.

Analytic Group	Total Ara-C Dosage	Total $T\beta_4$ Dosage	Total $N4\text{-}T\beta_4$ Dosage	Survival* Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
I	0	0.2 µg	0	15 (100%)	15 (100%)	15 (100%)	15 (100%)	15 (100%)	15 (100%)	15 (100%)	15 (100%)
II	15 mg	0	0	29 (100%)	29 (100%)	29 (100%)	19 (66%)	16 (55%)	14 (48%)	14 (48%)	14 (48%)
III	15 mg	2 µg	0	29 (100%)	29 (100%)	29 (100%)	28 (97%)	25 (86%)	21 (72%)	19 (66%)	19 (66%)
	15 mg	0.2 µg	0	29 (100%)	29 (100%)	29 (100%)	29 (100%)	29 (100%)	28 (97%)	28 (97%)	28 (97%)

Table I continued

Effect of the N-terminal tetrapeptide of $T\beta_4$ ($N4\text{-}T\beta_4$) or $T\beta_4$ on ara-C treated mice.

Analytic Group	Total Ara-C Dosage	Total $T\beta_4$ Dosage	Total $N4\text{-}T\beta_4$ Dosage	Survival* Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
IV	15 mg	0	2 µg	29 (100%)	29 (100%)	28 (97%)	25 (86%)	19 (66%)	18 (62%)	18 (62%)	17 (59%)
	15 mg	0	0.2 µg	29 (100%)	29 (100%)	29 (100%)	27 (93%)	26 (90%)	23 (79%)	23 (79%)	23 (79%)

*Survival is given as number of surviving mice followed by percent survival in parentheses.

What is claimed is:

1. A method of substantially inhibiting normal stem cell proliferation in a mammal which comprises administering a normal stem cell proliferation-inhibiting effective amount of $T\beta_4$ to said mammal.

2. The method of claim 1, wherein said mammal is human.

3. The method of claim 2, wherein the $T\beta_4$ is administered at a dosage from about 0.01 to about 2.0 mg per kg of body weight per day.

4. The method of claim 2, wherein the $T\beta_4$ is administered at a dosage from about 0.02 to about 0.2 mg per kg of body weight per day.

5. The method of claim 3, wherein the $T\beta_4$ is administered parenterally.

6. The method of claim 5, wherein the $T\beta_4$ is administered intravenously.

7. A composition which comprises a normal stem cell proliferation-inhibiting effective amount of $T\beta_4$ and a pharmaceutically acceptable carrier.

8. The composition of claim 7, wherein, for each unit dosage, the composition contains from about 0.01 to about 2.0 mg per kg of body weight per day of $T\beta_4$.

9. The composition of claim 7, wherein, for each unit dosage, the composition contains from about 0.02 to about 0.2 mg per kg of body weight per day of $T\beta_4$.

10. The composition of claim 8 which is in a form for parenteral administration, wherein the carrier is a sterile liquid carrier suitable for parenteral administration.

11. The composition of claim 10 which is in a form for intravenous administration.

12. The composition of claim 11, wherein the $T\beta_4$ is dissolved in a sterile isotonic saline solution.

13. A method for decreasing the toxicity of a toxic therapeutic composition in a mammal which comprises administering to said mammal a normal stem cell proliferation-inhibiting effective amount of $T\beta_4$.

14. The method of claim 13, wherein said mammal is human.

15. The method of claim 14, wherein the $T\beta_4$ is administered at a dosage from about 0.01 to about 2.0 mg per kg of body weight per day.

16. The method of claim 14, wherein the $T\beta_4$ is administered at a dosage from about 0.02 to about 0.2 mg per kg of body weight per day.

17. The method of claim 15, wherein the $T\beta_4$ is administered parenterally.

18. The method of claim 17, wherein the $T\beta_4$ is administered intravenously.

19. The method of claim 13, wherein said toxic therapeutic composition is an anti-bacterial agent.

20. The method of claim 13, wherein said toxic therapeutic composition is an anti-viral agent.

21. The method of claim 20, wherein said anti-viral agent is a nucleoside analog.

22. The method of claim 13, wherein said therapeutic composition is an anti-cancer agent.

23. The method of claim 22, wherein said anti-cancer agent is ara-C.

24. The method of claim 13, wherein said toxic therapeutic composition comprises at least two therapeutic agents.

25. The method of claim 13, wherein a therapeutically effective amount of the toxic therapeutic composition is administered in combination with $T\beta_4$.

26. The method of claim 25, wherein the toxic therapeutic composition and the $T\beta_4$ are administered substantially concurrently.

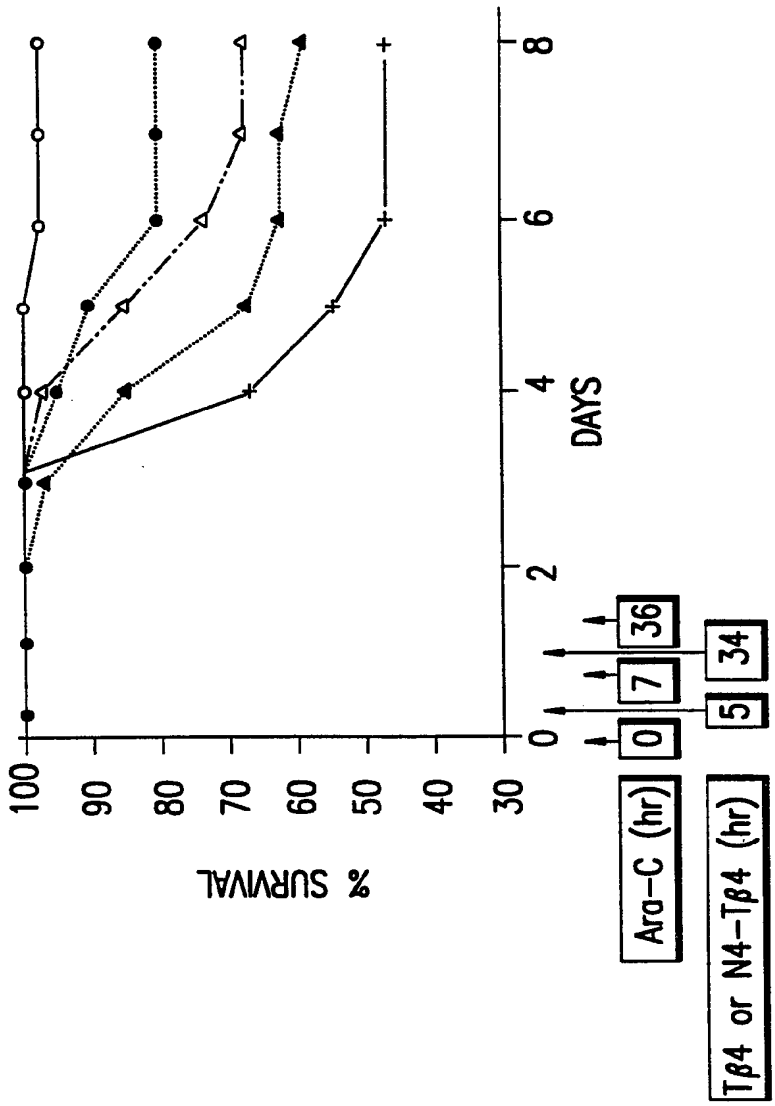
% SURVIVAL
100
90
80
70
60
50
40
30
0
2
4
6
8
DAYS
Ara-C (hr)
0
7
36
Tβ4 or N4-Tβ4 (hr)
5
34

FIG. 1

INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/US 94/04359

A. CLASSIFICATION OF SUBJECT MATTER

A 61 K 37/02

According to International Patent Classification (IPC) or to both national classification and IPC 5

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A 61 K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4 297 276 (GOLDSTEIN et al.) 27 October 1981 (27.10.81), claim 2; column 8, lines 25-46; column 12, line 25 - column 13, line 12. ----	1,3-13,15-18

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 94/04359

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-6,13-26
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-6,13-26 are directed to a method of treatment of the human or animal body (Rule 39.1(iv)PCT) the search has been carried out and based on the alleged efforts of the compounds.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANHANG

zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

ANNEX

to the International Search Report to the International Patent Application No.

ANNEXE

au rapport de recherche international relatif à la demande de brevet international n°

PCT/US 94/04359 SAE 90160

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The Office is in no way liable for these particulars which are given merely for the purpose of information.

La présente annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche international visée ci-dessus. Les renseignements fournis sont donnés à titre indicatif et n'engagent pas la responsibilité de l'Office.

Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
US A 4297276	27-10-81	keine - none - rien	